

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
27 June 2002 (27.06.2002)

PCT

(10) International Publication Number
WO 02/50251 A2

- (51) International Patent Classification⁷: C12N 1/36, C12M 3/02 (74) Agent: MAHONEY, John, W.; Bayer corporation, 800 Dwight Way, P.O. Box 1986, Berkeley, CA 94701 (US).
- (21) International Application Number: PCT/US01/49761 (81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.
- (22) International Filing Date: 18 December 2001 (18.12.2001)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data: 09/746,972 20 December 2000 (20.12.2000) US (84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
- (71) Applicant (*for all designated States except US*): BAYER CORPORATION [US/US]; 800 Dwight Way, P.O. Box 1986, Berkeley, CA 94701 (US).
- (72) Inventors; and (75) Inventors/Applicants (*for US only*): HEIDMANN, Rudiger [DE/US]; 6401 Shellmound Street, Apt. 8202, Emeryville, CA 94608 (US). MERED, Mokhtar [US/US]; 8 Rae Drive, Orinda, CA 94563 (US). MICHAELS, James, D. [US/US]; 1318 Bates Road, Oakland, CA 94610 (US). KONSTANTINOV, Konstantin [US/US]; 116 Foxglove Lane, Walnut, CA 94596 (US).
- Published:**
— *without international search report and to be republished upon receipt of that report*
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*



WO 02/50251 A2

(54) Title: A DEVICE AND METHOD FOR SEED-TRAIN EXPANSION OF MAMMALIAN CELLS

(57) Abstract: A device and method for seed-train expansion of a source of mammalian cells and their use in production of proteins expressed by the cells comprising the use of a dedicated inoculation bioreactor for expanding the cells prior to the transfer to a production bioreactor is disclosed. The novel inoculation bioreactor is designed to facilitate an improved method of mammalian cell seed-train expansion, and is distinguished by the presence of an "inoculation well" which communicates with the interior of the bioreactor and which facilitates the growth of mammalian cells for commercial seed-train expansion. The method comprises adding the cryopreserved cells to media within the inoculation well of the inoculation bioreactor, enabling the cells to grow to a predetermined concentration within the inoculation well by monitoring and adjusting the condition of the media and environment, and thereafter incrementally increasing the volume of the media within the reactor so that optimal cell growth is maintained.

A DEVICE AND METHOD FOR SEED-TRAIN EXPANSION OF MAMMALIAN CELLS.

RELATED APPLICATIONS

This Application claims priority of Application 09/746,972 filed on December 20, 2000, which was converted to a Provisional Application under 37 C.F.R. §1.53(c)(2) on December 14, 2001, which is herein incorporated by reference.

BACKGROUND OF THE INVENTION

Field: This invention relates to manufacturing scale mammalian cell culture technology and specifically to a new seed-train expansion method for mammalian cells. Cryopreserved cells are used to inoculate directly into a newly designed bioreactor, which serves as a seed source for production scale.

Background: The start of a new cell seed-train expansion for a production campaign for the expression of a mammalian cell culture system is a critical process step. Operational inconsistencies and errors often compromise the process, leading to significant delays and inoculum variability. In typical production protocols, a new seed train expansion begins from a 1-2 mL cryovial (master working cell bank, MWCB). The cell concentration of this container is usually in the range of 5 – 10 million cells/mL. The cells are thawed, washed to remove the cryoprotectant and then first cultured (seeded) in small tissue culture flasks. It is standard practice with mammalian cell cultures to use

inoculation densities of 0.5 to 1×10^6 cells/mL. Seeding cells at lower cell concentrations after cryopreservation may result in an extended lag phase prior to entering the growth phase, poor cell performance or even cell death. This is especially accentuated in the serum-free or even protein-free cell culture media that has become the *modus operandi* for current cell culture manufacturing.

The cells are sub-cultivated according to their specific growth rate (cell density), and are usually split into multiple cell culture flasks every 2 - 3 days. Once enough biomass is produced, the cells are expanded into larger cultivation bottles such as roller bottles, shake or spinner flasks. After enough cell mass is accumulated, a bioreactor, which becomes the seed culture for the production-scale vessel, is inoculated. This described seed-train expansion is general practice for several mammalian cell lines and is widely used in commercial production and academia. An overview of a commercial seed train expansion using T-flasks and spinner flasks is also given by *Whitaker et al., Journal of the American Chemical Society, pages 28-43, 1998.*

Typical scale-up protocols which take about four to six weeks to complete under optimal conditions are labor intensive and are susceptible to contamination and variability. The small scale conditions are not well defined – usually there is no set-point for pH and DO during the T-flask, roller bottle or shaker flask period, which may lead to more variability throughout the process and might compromise the quality of the inoculum and the product.

SUMMARY OF THE INVENTION

We have developed a novel bioreactor and method for the seed-train expansion of mammalian cells. This technology eliminates the use of tissue culture-flasks, roller bottles or shake flasks.

Our novel inoculation bioreactor is designed to facilitate an improved method of mammalian cell seed-train expansion, and is distinguished by the presence of an “inoculation well” which communicates with the interior of the bioreactor and which facilitates the growth of mammalian cells for commercial seed-train expansion.

Our method for seed-train expansion of cryopreserved cells comprises the use of a dedicated inoculation bioreactor having an inoculation well for expanding the cells prior to their transfer to a production bioreactor. More specifically, our method comprises adding the cryopreserved cells to media within the inoculation well of the inoculation bioreactor, enabling the cells to grow to a predetermined concentration within the inoculation well by monitoring and adjusting the condition of the media and environment, and thereafter incrementally increasing the volume of the media within the reactor so that optimal cell growth is maintained. Once the desired cell density and volume is achieved, the cells are transferred to a production bioreactor.

In a preferred embodiment, the inoculation bioreactor includes an “inoculation well” located at the base of the reactor chamber. This inoculation well is supplied with fermentation sensors (pH, dissolved oxygen, temperature, optical density, etc.) to assist in assuring optimal cultivation conditions. Most preferably, this inoculation well is adapted

to include an impeller drive by a continuously stirred tank reactor (CSTR) liquid agitation device.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 illustrates the prior art of seed-train expansion method.

Figure 2 shows the preferred method of this invention.

Figure 3 shows the concept of the inoculation well inoculation bioreactor.

Figure 4 shows a continuous perfusion culture performed in the 5 L "inoculation well" containing bioreactor, started with BHK cells cryopreserved in the 50 mL bag. The viable cell density (VCD [•]) and the viability profile [] are shown. Cell viability remained high throughout the culture. The target cell density of 20 million cells/mL at 5 L scale was reached within 12 days.

Figure 5 illustrates the influence of a DMSO washing step during the seed-train expansion of BHK cells. In the 12 L culture (open symbols) a DMSO washing step was performed were as the second 5 L culture (filled symbols) was directly inoculated without washing the cells. As indicated no negative effect was observed, cell viability as well as cell growth showed no difference

Figure 6 shows a continuous 12 L bioreactor culture, started with BHK cells cryopreserved in the 50 mL bag, no DMSO wash was performed before the inoculation. The viable cell density (VCD [•]) and the viability profile [] are shown. The target cell density of 20 million cells/mL at 12 L scale was reached within 14 days.

Figure 7 illustrates a 5 L culture of recombinant CHO cells, started with CHO cells cryopreserved in a 50 mL bag, no DMSO wash was performed before the inoculation. The viable cell density (VCD [•]) and the viability profile [] are shown. The target cell density was reached within 6 days.

Figure 8a illustrates two 5 L cultures started with rBHK cells cryopreserved in a 50 mL bag, a second culture in a 100 mL bag, no DMSO wash was performed before the inoculation. The viable cell density (VCD [•]) and the viability profile [] are shown. The target cell densities of 20 million cells/mL at 5 L scale was reached within 10 days in both cultures.

Figure 8b illustrates two 12 L cultures started with recombinant CHO cells cryopreserved in 50 mL and 100 mL bags, no DMSO wash was performed before the inoculation. The viable cell density (VCD [•]) and the viability profile [] are shown. No negative effect was noticed in both cultures.

SPECIFIC EMBODIMENTS

Materials and Methods

This seed-train expansion method was initially developed for a recombinant baby hamster kidney (BHK) cell line and a chinese hamster ovary (CHO) cell line expressing human proteins. Cells used for this invention were taken from 12 L perfusion bioreactor containing 20×10^6 cells/mL. The cells were cultivated and frozen in mammalian cell culture medium based on a commercially available DMEM/Ham's F12 formulation (1:1) manufactured by JRH (Lenexa, KS) or Life Technologies (Grand Island, NY) and supplemented with iron, Pluronic F68 (BASF, Parsippany, NJ), recombinant human insulin (Humulin, Eli Lilly, Indianapolis, IN), and essentially free of other proteins. The freezing medium contained 7.5% dimethylsulfoxide (Sigma, St. Louis, MO) as a cryoprotectant.

The cryopreservation bags (Cryocyte™ 250 mL or 500 mL, Nexell Therapeutics Inc. Irvine, CA) used for storage contained 50 - 100 mL of cell suspension and were frozen in a -40°C freezer (Revco, Asheville, NC) prior to transferring to a liquid N_2 freezer (Forma Scientific, OH). The cells were concentrated to approx. 40×10^6 cells/mL in 50 mL, or to 20×10^6 cells/mL in 100 mL to ensure an initial bioreactor cell density of 1 million cell/mL in 2 L volume. Cell densities of $0.5 - 1 \times 10^6$ cells/mL are commonly used to initiate a new seed-train expansion process.

The BHK cells in the cryopreservation bag were thawed using a 37°C water bath. The cell suspension was transferred to a centrifuge tube, diluted with fresh medium (1:1) and gently centrifuged at 1000 - 1200 rpm. The supernatant was discarded, the cell pellet resuspended in fresh medium and transferred to a sterile bottle to inoculate the "inoculation well" bioreactor. This wash step is used to remove most of the DMSO and is common practice in mammalian cell culture. Later, this DMSO washing step was eliminated and the cells were directly transferred into the bioreactor from the bag. (Elimination of the DMSO wash step allows operation and maintenance of a closed system, thereby reducing the opportunity for system contamination. See Example 2.)

The same technology was also used for recombinant CHO-cells.

Two different bioreactors were used. However, both of them had the common feature of being able to inoculate into a 2 L volume "inoculation well".

1. 5 L cultures were carried out in Applikon 7 L bioreactors (Schiedam, The Netherlands) equipped with a sintered stainless steel frit (Mott Metallurgical, Farmington, CT) for aeration. A cell separation device was used to retain cells within the vessel and to allow for operation in continuous perfusion mode. The reactor system is capable of supporting at least 20 million cells/mL in 5 L volume. pH, dissolved oxygen, temperature, and optical density were measured via probes that were submerged in culture when a volume of at least 2 L was used. The reactor is designed with a 2 L volume stainless steel inoculation well.

2. 12 L cultures were carried out in custom designed 15 L bioreactor. The stainless steel inoculation well of the reactor was the same (2 L volume) as in the 7 L vessel. However, a conical flange was used to extend the diameter and the volume of the top part (figure 3). Aeration was provided through a sintered stainless steel frit (Mott Metallurgical, Farmington, CT). A cell separation device was used for continuous perfusion technology. This reactor system is capable of supporting at least 20 million cells/mL in a 12 L working volume.

Both bioreactor systems were controlled using a B. Braun DCU (Digital Control Unit, B. Braun International, Melsungen, Germany) under operating conditions as reported elsewhere.

The relationship between the size and cell density of the used cryo-container and the inoculation well ($V_{IR \min}$) of the inoculation reactor can be determined by following formula:

$$V_{IR \min} = \frac{X_{bag} * V_{bag}}{X_{IR start}}$$

where X_{bag} is the cell density in the cryo-container and V_{bag} the volume. $X_{IR start}$ is the desired starting cell density in the inoculation well of the inoculation reactor, usually 1×10^6 cells/mL.

The final volume of the inoculation reactor ($V_{IR max}$) depends on the volume of the production reactor that will be used and can be determined by the formula:

$$V_{IR \max} = \frac{X_{PR} * V_{PR}}{X_{IR}}$$

where X_{PR} is the targeted initial cell density in the production bioreactor (usually $1 - 10^6$ cells/mL), V_{PR} the volume of the production reactor, and X_{IR} the final cell density in the inoculum bioreactor. The inoculation well of the bioreactor will normally be small (in the present examples a 2L inoculation well was used) and the volume may be increased by increasing the diameter and the height of the bioreactor. Since $V_{IR \max}$ is not limiting, the same "inoculation well" concept can be applied to the design of larger bioreactors. (see figure 3).

The cultures carried out in the 7 and 12 L systems had an initial volume of 2 L. This is the minimum volume necessary to submerge the bioreactor's pH, dissolved oxygen electrodes, as well as the temperature sensor. Aeration is carried out using headspace aeration just after inoculation. The volume is increased stepwise to keep the cell density between 0.8 and 1.2×10^6 cells/mL. Oxygenation via gas sparging is used once the volume reaches four liters. The cell-specific perfusion rate was maintained at $0.5 - 0.7$ nL/cell/day at all times.

Off-line sampling was performed daily to determine cell and metabolite concentrations. Cell counts and viability were determined using a hemacytometer and

the trypan blue exclusion method. A YSI analyzer (Yellow Springs Instruments) was used to measure glucose, lactate, glutamate and glutamine concentrations of samples. LDH and ammonia were measured using a Kodak Biolyzer (Kodak Instruments, NY). A NOVA blood gas analyzer (Nova Biomedical, Waltham, Mass.) was used to measure the dissolved CO₂ level and to check pH and dissolved oxygen values. Samples were analyzed for rFVIII activity by the one stage coagulation method. The product quality was determined by an in-house developed Western Blot assay.

EXAMPLE 1

Figure 4 shows a 12 L perfusion culture. 50 mL of cell suspension cryopreserved in an EVA bag was used for inoculation. The cells were washed with fresh medium to remove the DMSO before inoculation. The culture was at an initial volume of 2 L with headspace aeration. The cell viability exceeded 95% throughout the process and the volume was increased stepwise based on cell density. Gas sparging was used once the volume was at 4 L. Continuous medium perfusion was started one day after the volume reached 12 L. The perfusion rate was kept at 0.5 – 0.7 nL/cell/day. The target cell density of 20×10^6 cells/mL was reached after 11 days. The seed-train expansion experiment clearly demonstrates the elegance of this new expansion technology. No intermediate cell culture flasks were used, the risk of possible contamination was dramatically

reduced, the media and environment were externally controlled, and as a result the time to start a production bioreactor was reduced by at least 70%.

EXAMPLE 2

A necessity of the DMSO removal step (wash/dilution) before the inoculation was evaluated. In this evaluation, two reactors were started in parallel from the same 50 mL bag freeze lot.

- 12 L reactor where a DMSO washing step was performed.
- 5 L reactor where a DMSO wash was omitted.

Both cultures were run under the same conditions (volume increase steps, headspace aeration and sparging rates etc.). The 5 L culture reached the target cell density of 20×10^6 cells/mL within 11 days, the 12 L culture one day later. Figure 5 shows a comparison of these two cultures. No difference in cell growth or viability was noticed. Elimination of the DMSO wash step further streamlines the seed-train expansion process (from cell thaw onwards) and allows for system closure throughout.

EXAMPLE 3

Figure 6 shows the time profile of a culture performed in the 12 L "inoculation well" reactor. 50 mL of cell suspension, cryopreserved in a bag, was used for inoculation

without a DMSO wash. The target cell density of 20×10^6 cells/mL was reached after 16 days.

EXAMPLE 4

Figure 7 shows the time profile of a CHO culture performed in the 5 L bioreactor. 50 mL of cryopreserved cells in a bag was used for inoculation. No DMSO washing step was performed. The target cell density – here 10×10^6 cells/mL were reached within 6 days.

EXAMPLE 5

Figure 8a and 8b show the time profiles of two BHK and two CHO cultures performed in the inoculation bioreactors. 50 and 100 mL cryobags were used for inoculation, no DMSO washing step was performed in all four cultures. No negative effect was noticed using either 50 mL or 100 mL cryobags.

SUMMARY

In general, existing seed-train expansion methods for mammalian cell culture are labor intensive and susceptible to microbial contamination due to the use of multiple

cultivation vessels such as T-flasks, roller-bottles and spinner flasks. A new seed-train expansion method was developed utilizing 50 mL or 100 mL EVA cryopreservation bags that are used to inoculate directly into a custom built "inoculation well" in a bioreactor. This reactor serves as the inoculation source for a production scale system. This methodology completely eliminates the use of any T-flasks, roller bottles or spinner flasks during the scale-up. A procedure for sizing and design of the "inoculation well" reactor is proposed. Following this procedure, the method can be applied for the direct inoculation of any scale production reactor. Cryopreserved cells were used in these examples, but cryopreservation is not a requirement of this technology. Mammalian cells at standard temperatures can be substituted for the cryopreserved cells and the effectiveness and utility of the system would remain the same. Similarly, the size of the bioreactor can be adjusted, so that as long as the concepts of an inoculation well and the method of use are maintained.

The Examples presented above are provided to illustrate one organization design for the invention. Accordingly, it is intended that the scope of this invention should be limited only by the claims below.

CLAIMS

We claim:

1. A method of mammalian cell seed-train expansion comprising:
 - a) providing a bioreactor having an inoculation well,
 - b) delivering a suspension of mammalian cells to media within the inoculation well,
 - c) controlling environmental conditions and composition of said media so that cell growth within the inoculation well is optimized,
 - d) growing the mammalian cells until a predetermined cell density is reached within said well, and
 - e) increasing the media volume incrementally while maintain optimum environmental conditions and environmental growth conditions until the bioreactor is filled to a predetermined volume and cell density.
2. The method of claim 1, wherein the mammalian cells are cryopreserved cells.
3. The method of Claim 1, wherein the mammalian cells are selected from the group consisting of chinese hamster ovary cells and baby hamster kidney cells.
4. The method of claim 1, wherein the cells are obtained from a cryobag.

5. The method of claim 1, wherein the cells are obtained from a cryovial.
6. An improved fermentation bioreactor comprising:
 - a) a bioreactor, said bioreactor defining a chamber,
 - b) said chamber having exterior and interior sides, a top member, wall member, and base member, said base member having an orifice,
 - c) an inoculation well, said well defining a chamber having exterior and interior sides, top and base members, a wall member, and
 - d) said well further including an orifice in said top member joined to said bioreactor orifice, so that a channel is formed between the well chamber and the bioreactor chamber to permit unrestricted movement there between.
7. An improved bioreactor as recited in Claim 6, in which the inoculation well chamber is provided with sensors selected from the group consisting of: pH sensor, oxygen sensor, temperature sensor, and optical sensor.
8. An improved bioreactor as recited in Claim 6, in which the inoculation well is provided with a communication orifice through the wall of said well to permit the introduction of media and agents into the well chamber.

9. An improved bioreactor as recited in Claim 6, in which the inoculation well is provided with a mechanical arm to permit stirring of the contents of said well.

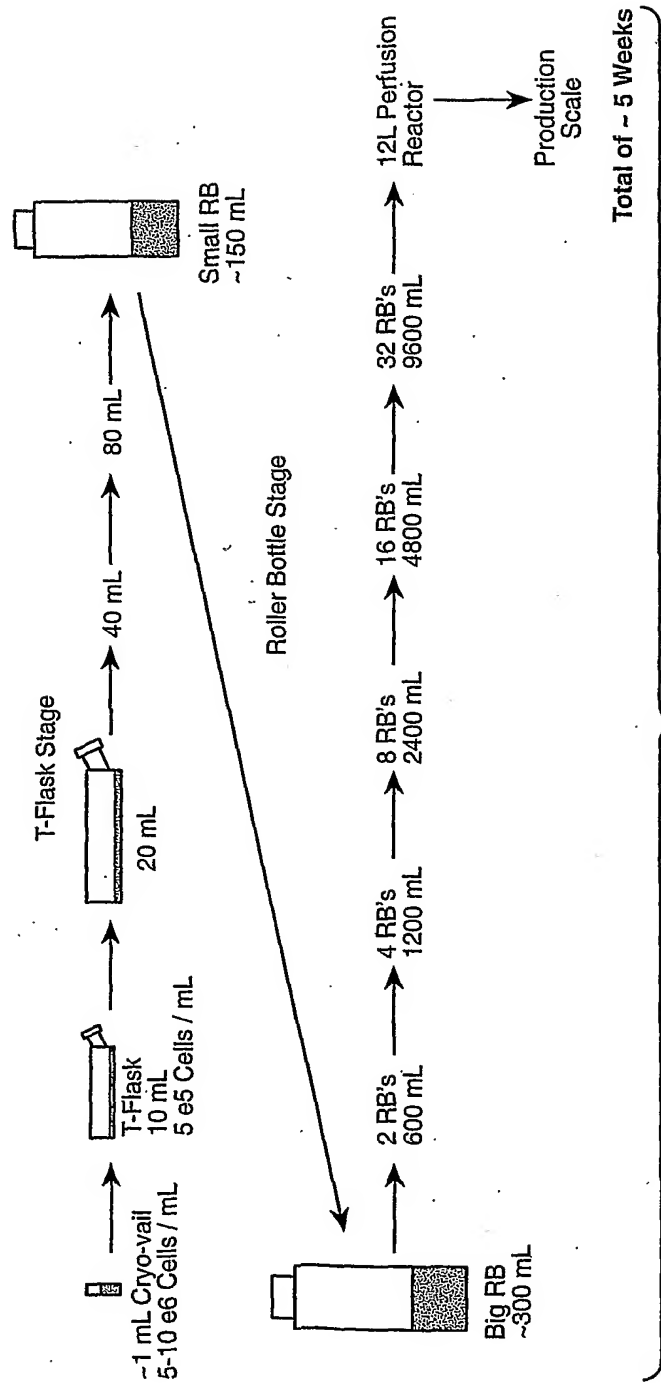


FIG. 1

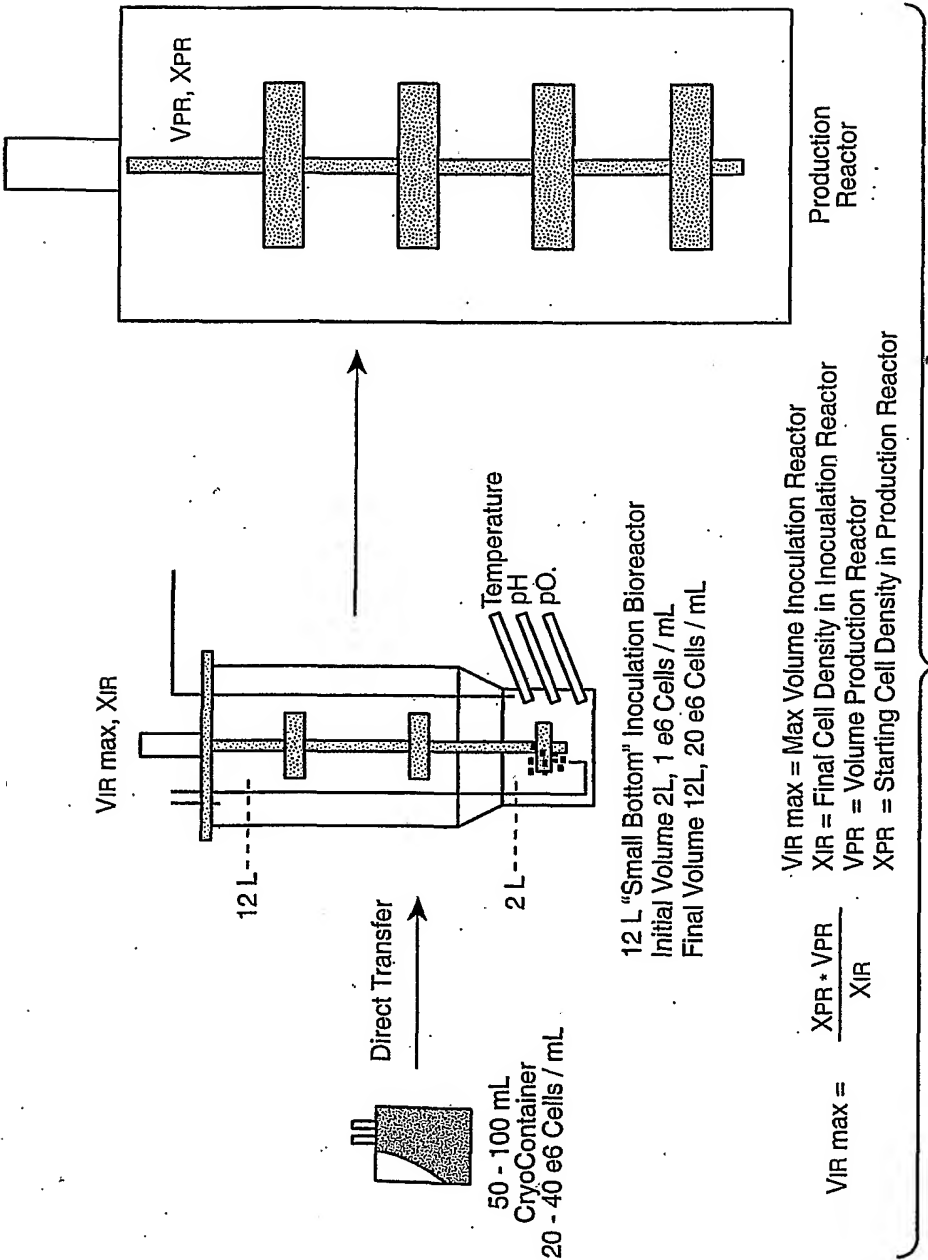
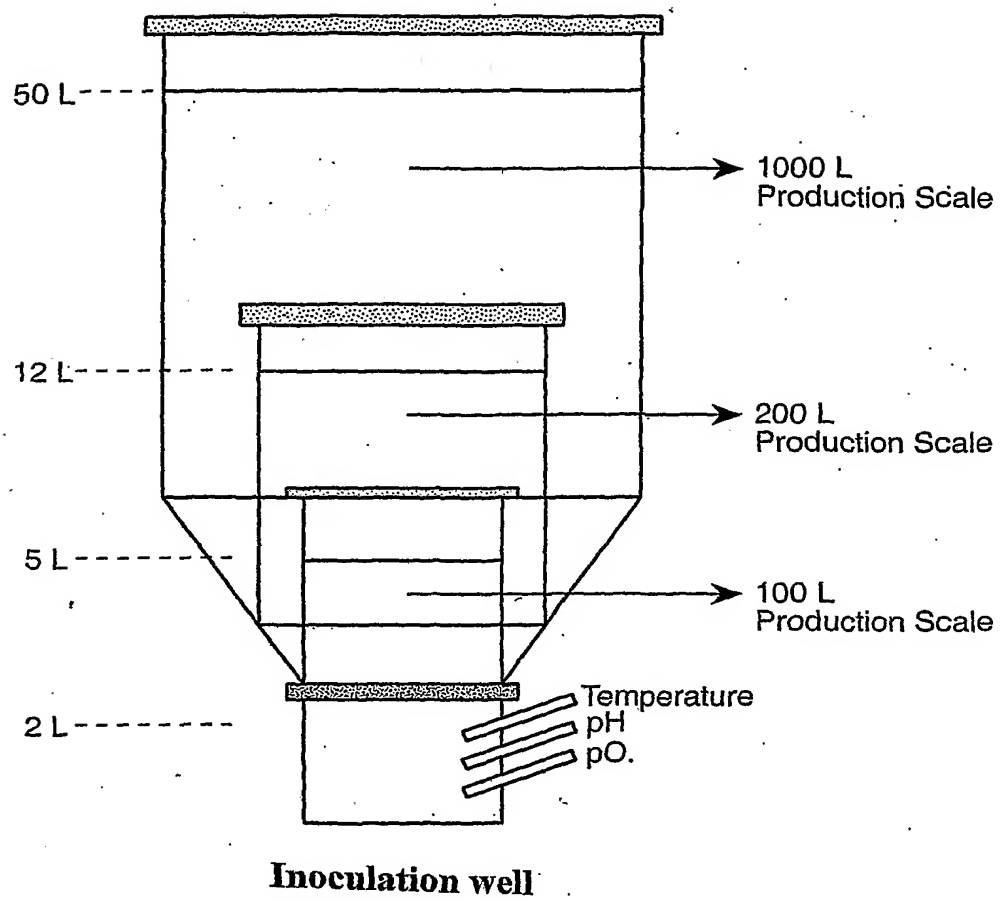


FIG. 2

**FIG. 3**

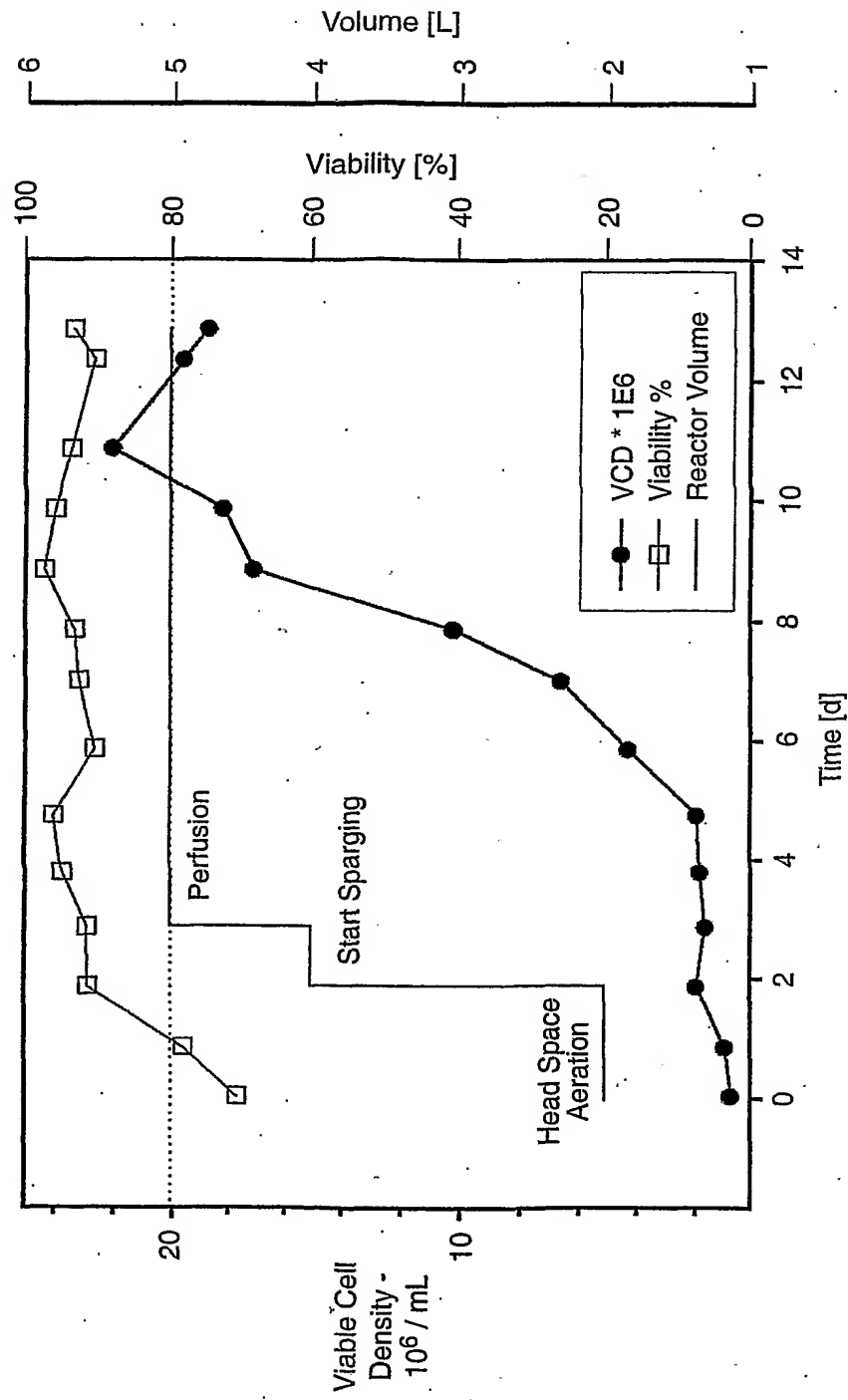


FIG. 4

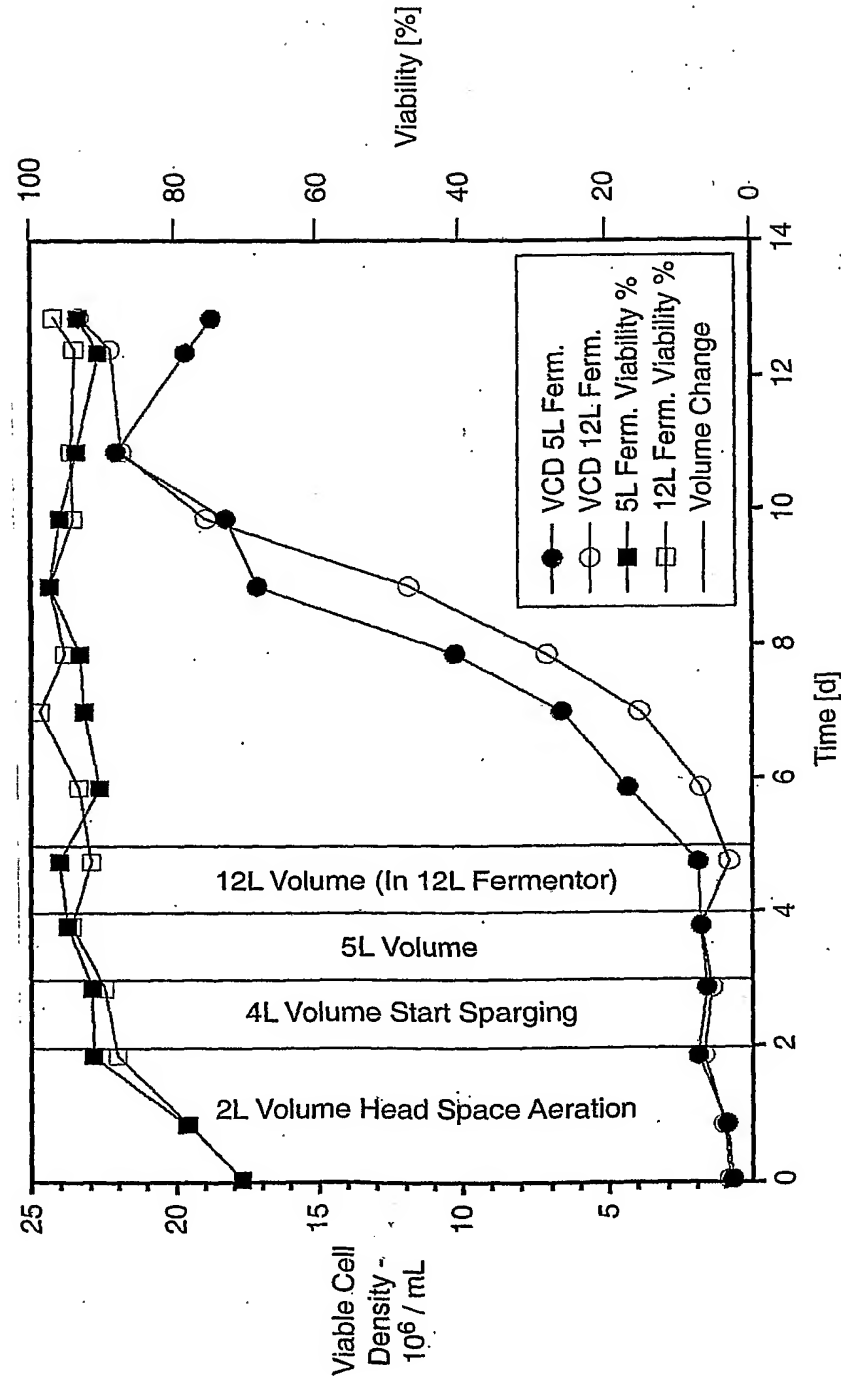


FIG. 5

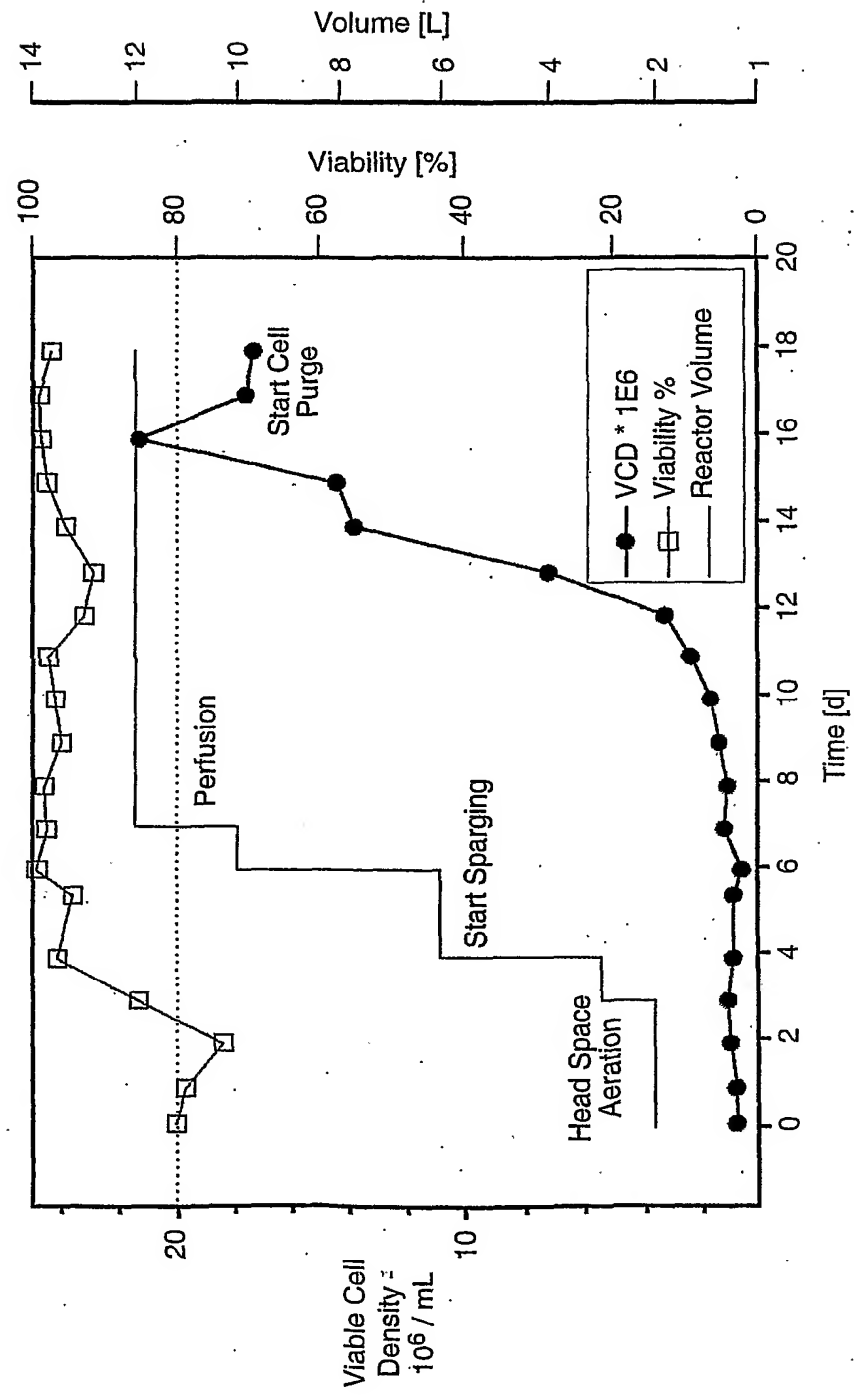


FIG. 6

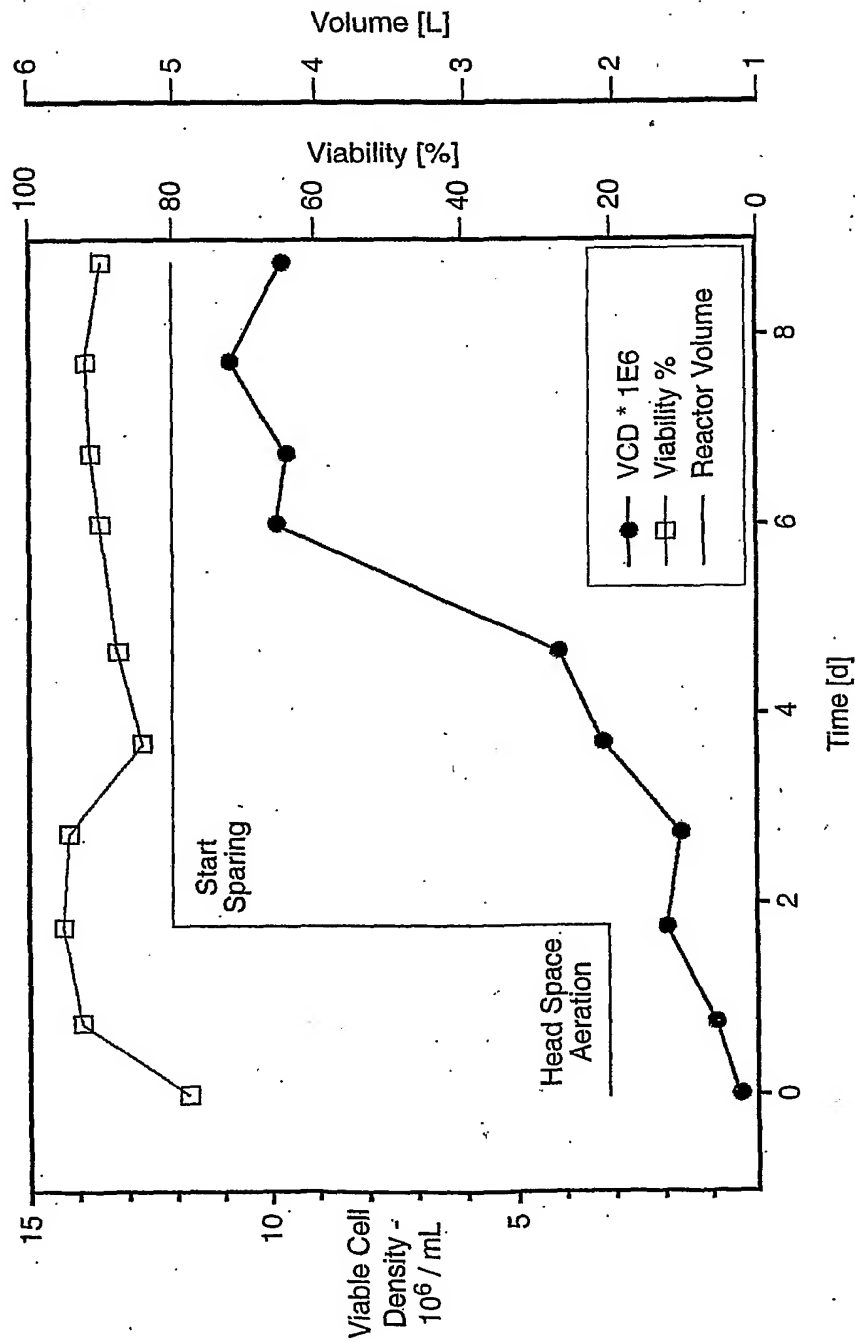


FIG. 7

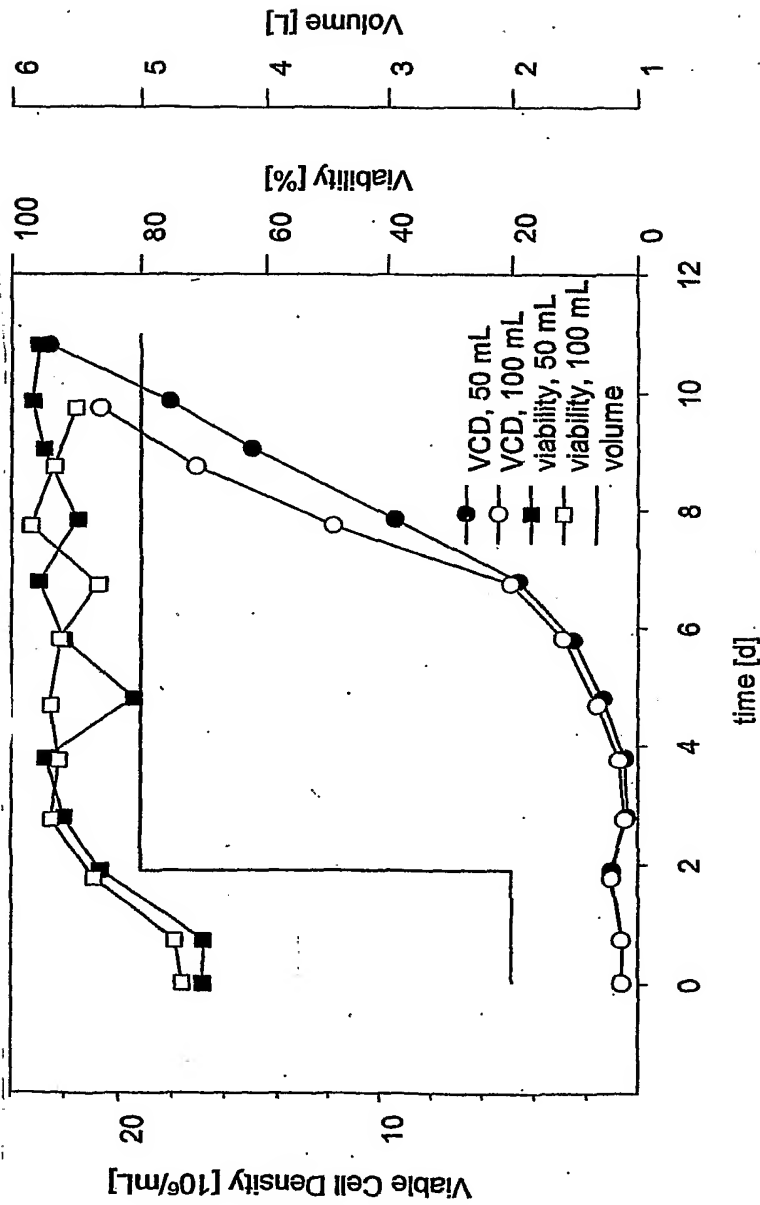


FIG. 8a

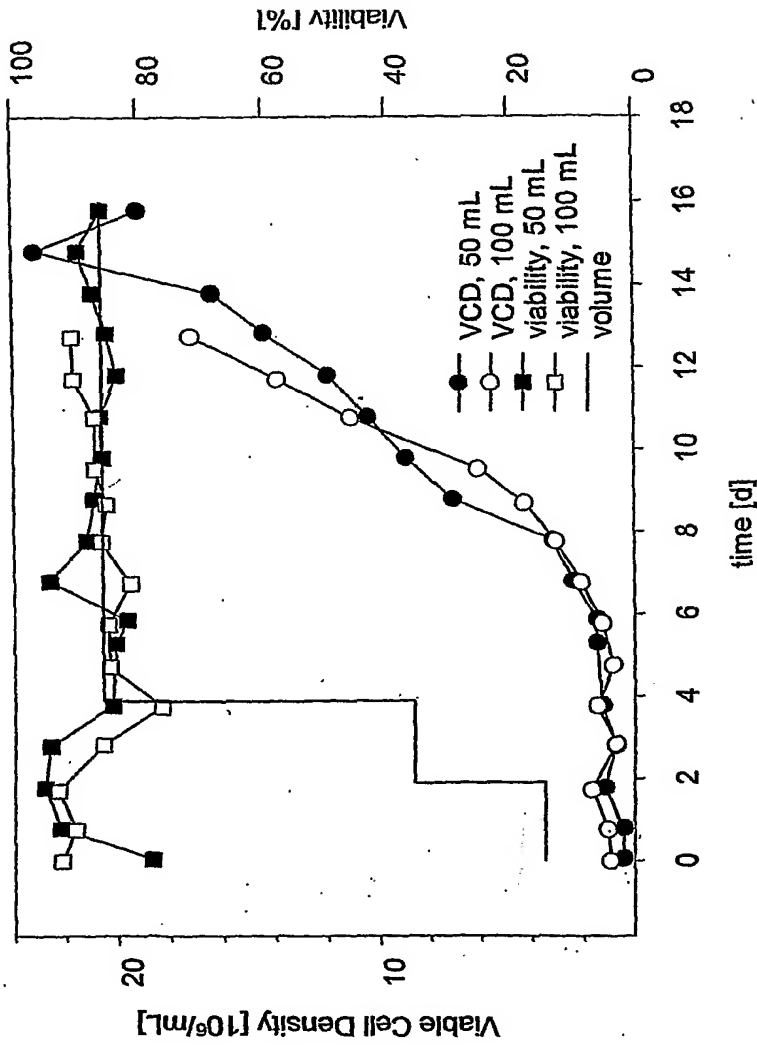


FIG. 8b

DERWENT-ACC-NO: 2002-500626**DERWENT-WEEK:** 200769*COPYRIGHT 2008 DERWENT INFORMATION LTD*

TITLE: Improved fermentation bioreactor
comprising a bioreactor defining
chamber useful for mammalian cell (e.
g., a recombinant baby hamster kidney
cell line and Chinese hamster ovary
cell line) seed-train expansion

INVENTOR: HEIDMANN R; KONSTANTINOV K ; MERED M ;
MICHAELS D ; MICHAELS J ; MICHAELS J D

PATENT-ASSIGNEE: BAYER CORP[FARB] , BAYER PHARM CORP
[FARB]

PRIORITY-DATA: 2000US-746972 (December 20, 2000)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE
WO 0250251 A2	June 27, 2002	EN
AU 200232726 A	July 1, 2002	EN
EP 1395647 A2	March 10, 2004	EN
JP 2005502306 W	January 27, 2005	JA
AU 2002232726 A8	October 20, 2005	EN
EP 1395647 B1	November 22, 2006	EN
DE 60124780 E	January 4, 2007	DE
ES 2276853 T3	July 1, 2007	ES
DE 60124780 T2	October 11, 2007	DE

DESIGNATED-STATES: AE AG AL AM AT AU AZ BA BB BG BR BY
 BZ CA CH CN CR CU CZ DE DK DM DZ EE
 ES FI GB GD GE GH GM HR HU ID IL IN
 IS JP KE KG KP KR KZ LC LK LR LS LT
 LU LV MA MD MG MK MN MW MX MZ NO NZ
 PL PT RO RU SD SE SG SI SK SL T J
 TM TN TR TT TZ UA UG US UZ VN YU ZA
 ZW AT BE CH CY DE DK EA ES FI FR GB
 GH GM GR IE IT KE LS LU MC MW MZ NL
 OA PT SD SE SL SZ TR TZ UG ZM ZW AL
 AT BE CH CY DE DK ES FI FR GB GR IE
 IT LI LT LU LV MC MK NL PT RO SE SI
 TR DE ES FR GB IT

APPLICATION-DATA:

PUB-NO	APPL- DESCRIPTOR	APPL-NO	APPL-DATE
WO2002050251A2	N/A	2001WO- US49761	December 18, 2001
DE 60124780E	N/A	2001DE- 624780	December 18, 2001
DE 60124780T2	N/A	2001DE- 624780	December 18, 2001
EP 1395647A2	N/A	2001EP- 992263	December 18, 2001
EP 1395647B1	N/A	2001EP- 992263	December 18, 2001
EP 1395647A2	N/A	2001WO- US49761	December 18, 2001
JP2005502306W	N/A	2001WO- US49761	December 18, 2001
EP 1395647B1	N/A	2001WO- US49761	December 18, 2001
DE 60124780E	N/A	2001WO- US49761	December 18, 2001
DE 60124780T2	N/A	2001WO- US49761	December 18, 2001

AU 200232726A	N/A	2002AU-032726	December 18, 2001
AU2002232726A8	N/A	2002AU-232726	December 18, 2001
JP2005502306W	Based on	2002JP-552128	December 18, 2001

INT-CL-CURRENT:

TYPE	IPC DATE
CIPP	C12M1/36 20060101
CIPP	C12M1/36 20060101
CIPP	C12M3/00 20060101
CIPS	C12M1/36 20060101
CIPS	C12M3/02 20060101
CIPS	C12M3/02 20060101
CIPS	C12M3/02 20060101
CIPS	C12N5/06 20060101

ABSTRACTED-PUB-NO: WO 0250251 A2**BASIC-ABSTRACT:**

NOVELTY - An improved fermentation bioreactor (I) comprising a bioreactor (BR) defining chamber (BDC), top member, wall member, and base member (BM), where BM having an orifice, an inoculation well, well defining a chamber having exterior and interior sides, top and base members, wall member, and the well includes orifice in top member joined to BR orifice, so that a channel is formed between well chamber and BDC.

DESCRIPTION - (I) Comprises a BDC, the chamber having exterior and interior sides, a top member, wall member,

and BM, where BM having an orifice, an inoculation well, a well defining a chamber having exterior and interior sides, top and base members, a wall member, and the well further includes an orifice in the top member joined to BR orifice, so that a channel is formed between the well chamber and BDC to permit unrestricted movement between them.

USE - (I) Is useful for mammalian cell seed-train expansion, by providing BR having an inoculation well, delivering a suspension of mammalian cells to media within the inoculation well, controlling environmental conditions and compositions of the media so that cell growth within the inoculation well is optimized, growing the mammalian cells until a predetermined cell density is reached within the well, and increasing the media volume incrementally while maintain optimum environmental conditions and environmental growth conditions until BR is filled to a predetermined volume and cell density, where the mammalian cells are cryopreserved cells. The mammalian cells are selected from Chinese hamster ovary (CHO) cells and baby hamster kidney (BHK) cells. The cells are obtained from a cryobag or cryovial (claimed).

ADVANTAGE - (I) Eliminates the use of tissue culture-flask, roller bottles or shake flask and is also designed to facilitate an improved method of mammalian cell seed-train expansion, and is distinguished by the presence of an inoculation well which communicates with the interior of (I) and which facilitates the growth of mammalian cells for commercial seed-train expansion.

EQUIVALENT-ABSTRACTS:

INSTRUMENTATION AND TESTING

Preferred Bioreactor: In (I), the inoculation well chamber is provided with sensors selected from pH sensor, oxygen sensor, temperature sensor, and optical

sensor. The inoculation well is provided with a communication orifice through the wall of the well to permit the introduction of media and agents into the well chamber. The inoculation well is provided with a mechanical arm to permit stirring of the contents of the well.

The seed-train expansion method was developed for a recombinant baby hamster kidney (BHK) cell line and Chinese hamster ovary (CHO) cell line expressing human proteins. Cells were taken from 12 L perfusion bioreactor. The cells were cultivated and frozen in mammalian cell culture medium and supplemented with iron, pluronic F68, recombinant human insulin, and essentially free of other proteins. The cryopreservation bags used for storage contained 50-100 ml of cell suspension and were frozen in a -40 degreesC freezer. The cells were concentrated to approximately 40×10^6 cells/ml in 50 ml, or to 20×10^6 cells/ml in 100 ml to ensure an initial bioreactor cell density of 1 million cell/ml in 2 L volume. Cell densities of $0.5-1 \times 10^6$ cells/ml were commonly used to initiate a new seed-train expansion process.

The BHK cells in the cryopreservation bag were thawed. The cell suspension was transferred to a centrifuge tube, diluted and centrifuged. The cell pellet resuspended in fresh medium and transferred to a sterile bottle to inoculate the inoculation well bioreactor. This wash step was used to remove most of the DMSO and is common practice in mammalian cell culture.

Later, this DMSO washing step was eliminated and the cells were directly transferred into the bioreactor from the bag. 12 L cultures were carried out in custom designed 15 L bioreactor. A conical flange was used to extend the diameter and the volume of the top part. Aeration was provided through a sintered stainless steel frit. A cell separation device was used for

continuous perfusion technology. This reactor system was capable of supporting at least 20 million cells/ml in a 12 L working volume.

TITLE-TERMS: IMPROVE FERMENTATION COMPRISE DEFINE
CHAMBER USEFUL MAMMAL CELL RECOMBINATION
BABY HAMSTER KIDNEY LINE CHINESE OVARY
SEED TRAIN EXPAND

DERWENT-CLASS: B04 D16

CPI-CODES: B04-F01; B04-F02; B11-C08E1; B11-C09;
D05-A03; D05-H02; D05-H08; D05-J;

CHEMICAL-CODES: Chemical Indexing M1 *01*
Fragmentation Code M423 M424 M720 M740
N104 N105 N136 N137 Q233 Specific
Compounds RA00GT Registry Numbers
200757 200799

Chemical Indexing M6 *02*
Fragmentation Code Q233 R501 R502

SECONDARY-ACC-NO:

CPI Secondary Accession Numbers: 2002-141842